BOTANICAL GAZETTE

JULY, 1904

SPERMATOGENESIS AND OOGENESIS IN EPHEDRA TRIFURCA.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. LIX.

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(WITH PLATES I–V)

THE Gnetales are exceedingly important from a morphological standpoint because of many points of contact with angiosperms. That they have not received the attention their character warrants is probably due to the difficulty encountered in obtaining material suitable for critical morphological study.

Ephedra, comprising about twenty species, is confined to the warmer arid regions of the northern hemisphere, and is evidently more nearly related to the Coniferales than is either Tumboa or Gnetum.

Important morphological literature dealing with Ephedra is extremely scanty. In 1872 Strasburger published an account of *Ephedra altissima* and *E. campylopoda*, dealing with the development of the microsporangiate and megasporangiate strobili. In 1879 he described stages in the development of the embryo in *E. altissima*. Jaccard ('94) described in a fragmentary way *E. helvetica*, giving most attention to spermatogenesis. He also gave some attention to fertilization and early stages of embryogeny.

The present study was undertaken with the hope of being able to follow in a fairly complete way the life-history of *E. trifurca* Torr. This account, dealing with spermatogenesis and oogenesis, is to be followed shortly by another dealing with fertilization and embryogeny.

METHODS.

Material was collected in the vicinity of Mesilla Park, N. M., from December 20, 1902, to May 11, 1903. The second collection was made one month after the first; and as development became more rapid collections were made at intervals of four days. The strobili, attached to a short piece of the stem, were packed in wet cotton, and on reaching the laboratory four days later were placed in a moist chamber to enable them to recover turgescence. That fixation immediately after removal from the tree is not absolutely necessary is shown by nuclei in all stages of division. Further treatment did not differ essentially from approved methods of microtechnique.

THE STAMINATE STROBILUS.

E. trifurca is monosporangiate, and the staminate as well as the ovulate strobili are borne in whorls around the nodes of the stem. Exceptional instances were noted in which the strobili were bisporangiate (*fig. 1*). Strasburger figures such a strobilus in *E. campylopoda*, and refers to it as an abnormal inflorescence. In another instance two ovules were present in a staminate strobilus of *E. trifurca*, although one is the usual number in the ovulate strobilus. This last, however, has many exceptions.

Shaw ('96) reports a strobilus of *Sequoia sempervirens* in which the upper part was ovulate and the lower staminate. Dickson ('60) observed the same thing in *Picea excelsa*. Coulter and Chamberlain ('01) figure strobili of Abies which are staminate at the apex and base and ovulate between. Goebel ('01) observed that in *Pinus maritima* the microsporangia were at the base of the strobilus and the megasporangia above. In the middle region he found rudimentary ovuliferous scales in the axils of the microsporophylls. In Tumboa the flowers are functionally monosporangiate, but in the center of a whorl of stamens there is a single functionless ovule with a spirally coiled micropyle. This seems to indicate that at a not remote period of its history the flowers were perfect. Ephedra, however, appears to have gone a step farther, and has become wholly monosporangiate; and the occasional bisporangiate strobili are reversions. It seems that, instead of regarding such occasional strobili as abnormal, it is better to consider them as atavistic; as pointing back to a bisporangiate

ancestry. Atavistic tendencies should not be lightly passed over, for it is from such reversions that we may expect valuable hints as to previous conditions.

THE MICROSPORANGIUM.

In the first material collected, December 20, 1902, the group of cells which gives rise to the staminate flower is shown in *fig. 2*. No archesporial cells are yet distinguishable either by size or staining reactions, nor is the beginning of the perianth visible. Cell division is proceeding quite rapidly, and there is apparently considerable activity throughout the winter months.

A month later the perianth is quite well developed (*fig. 3*). The cells immediately beneath the epidermis are about the same size as the adjacent ones, and as yet no differentiation into archesporium can be recognized. At the base of the strobili the flowers are much farther advanced than at the apical region. Later in the season all stages from rudimentary sporangia to mother-cells may be found in the same strobilus.

Fig. 4 shows a later stage in the development of an anther taken from the base of a strobilus. There is no positive evidence that the archesporium rises from a single hypodermal cell, but such is probably the case. The primary wall layer divides periclinally, giving rise to the wall layer and tapetum. The wall-cells do not divide periclinally, all divisions being anticlinal (*figs. 5, 6*). The sporogenous cells do not divide in any definite plane (*fig. 5*). The stages shown in *fig. 5* were common in basal sporangia February 9, 1903.

Fig. 6 shows a more advanced stage of the sporangium. The wall layer and tapetum are completely separated, and the sporogenous cells have ceased to divide; in fact, they are spore mother-cells which have not yet taken on the appearance which is characteristic of older mother-cells (February 15, 1903). A little later the wall-cells become flattened by the growth of the mother-cells and the tapetum. No further anticlinal division of the wall-cells takes place after they begin to be flattened. They become much stretched by the further growth of the adjacent cells. The tapetal cells increase in size and stain intensely, this last because of the presence of food in large quantities. *Fig. 7* shows a more advanced stage. The wall-cells are

beginning to disintegrate, the tapetal cells are increasing in size and dividing anticlinally, and the mother-cells are in the resting condition (February 15, 1903).

In gymnosperms the number of wall layers varies considerably. In Cycadales, Lang ('97) found the wall of Stangeria to consist of from three to six layers; in Ginkgoales they number from four to seven. Chamberlain ('98) found the wall of *Pinus Laricio* to be almost constantly three-layered. Coker ('02) found three wall layers in Podocarpus, the cell-walls of which are very thin and ultimately collapse; and in Taxodium he found a single layer. In *E. trifurca* the wall is a single layer of cells, and Strasburger found the same condition in the species studied by him.

In *E. trifurca* numerous instances were observed in which individual tapetal cells were not distinguishable from adjacent mother-cells. This seems to indicate that the tapetum is potentially sporogenous, and by virtue of its position has become sterile. With the appearance of the mother-cell the history of the sporophyte ends.

In general the resting stage of the microspore mother-cell in gymnosperms is long. Chamberlain ('98) observed mother-cells in *Pinus Laricio*, *Cupressus Lawsoniana*, and *Taxus baccata canadensis* in October. The reduction division occurred about May 1, thus giving a resting period of about seven months. In Ephedra the first observed reduction division was on March 12, giving a resting period of about one month.

At the time of the reduction division the cells of the wall layer are reduced to nuclei, scarcely a trace of cytoplasm being present. The cells of the tapetal layer become conspicuously vacuolated and their nuclei much enlarged. The nuclei become usually about four times the volume of those of the epidermal cells (*fig. 7*). Two or more nuclei are present in many tapetal cells at the time tetrads are formed. These last divisions appear to be amitotic, and nuclei in all stages, from the dumb-bell stage to complete separation, can be seen. At this time the tapetal cells, especially those nearest the bottom of the loculi, become enormously distended and very vacuolate (*fig. 14*). Soon afterwards they become a flattened plate and disappear.

THE REDUCTION DIVISION AND MALE GAMETOPHYTE.

As has been said, the microspore mother-cell remains in the resting stage about one month. The mother-cells up to the late prophase are filled with starch, which now quickly disappears. There does not at all times seem to be uniformity in the stages of division in the cells of a loculus. Instances were observed in which all the cells of a loculus were in the same phase of division. Again, those in the upper part of a loculus were in synapsis, those at the bottom had formed tetrads, while all intermediate stages were between.

The spirem segments into twelve chromosomes (not all of which are shown in *fig. 8*), which as they come to lie in the equatorial plane of the nucleus are short and thick, closely massed, and can be counted only with extreme difficulty. The result of repeated countings made in various stages of the first division, as well as in the second, leaves no doubt that the gametophyte number is twelve. Jaccard reports eight in *E. helvetica.*

Twelve appears to be the prevailing number of chromosomes in the gametophytes of gymnosperms. Three exceptions to this statement are to be noted: Overton ('93) reports eight for *Ceratozamia mexicana;* Strasburger ('04) finds eight in *Taxus baccata;* the other is *E. helvetica,* with eight according to Jaccard. Dixon ('98) reported eight chromosomes for *Pinus sylvestris,* but Blackman ('98) and Miss Ferguson ('01) have shown beyond doubt that the number in this species is also twelve.

Each chromosome apparently consists of four rods lying in extremely close contact. In the heterotypic division (*fig. 9*) the chromosome divides longitudinally, the ends opening out to form the X, Y, V, and O forms which are characteristic of the heterotypic division in higher plants. A membrane (*figs. 11, 12*) begins to form between the daughter-nuclei, as if spores of the bilateral type are to result, but in the great majority of cases the membrane wholly disappears, and the spores are of the tetrahedral type; although instances were noted in which they are probably bilateral. The second division in the pollen mother-cell is homotypic (*fig. 13*) and immediately follows the heterotypic division. In this second division longitudinal splitting of the chromosomes can be seen with little difficulty. The J form is quite conspicuous. As the chromosomes separate, in

many instances they become quite irregular, being stretched almost to the point of breaking. It is quite possible that irregular numbers of chromosomes, which are occasionally reported in plants, may have originated by the breaking of an individual chromosome. The microspores, still within the wall of the mother-cell, quickly assume an oval form (*fig. 14*).

After a brief period of rest (*fig. 15*) the nucleus of the microspore divides, and the first prothallial cell is formed. It has been determined beyond a reasonable doubt that a wall is laid down between the two nuclei (*figs. 16, 17*), although it is extremely difficult to differentiate. The prothallial cell is pressed closely against the end of the microspore by the growth of the other cell, its nucleus usually taking the meniscus form (*figs. 17–22*). The other cell, still remaining at the center of the spore, enlarges, and dividing (*fig. 17*) gives rise to the second prothallial cell and the antheridium initial (*fig. 18*). The antheridium initial does not appear to be separated from the second prothallial cell by a wall, but both nuclei remain in the same mass of cytoplasm which originally surrounded the nucleus before division (*fig. 18*). Here again arises a great difficulty in making conclusive observations. It may be that a wall is laid down and almost immediately resorbed, as Juel ('00) has shown to be the case in tetrad formation in *Carex acuta*, where a cell plate is formed and immediately resorbed, leaving the tetrad nuclei free within the wall of the mother-cell. It is conceivable that such a wall may be laid down, for it must be remembered that the entire prothallial region of gymnosperms is undergoing modification, in that the prothallial cells are either more or less ephemeral or altogether wanting; and that when cells are becoming obsolescent the wall is the first to disappear, leaving the two nuclei free within the common cytoplasm; next, the nucleus occasionally fails to divide; and finally no division takes place at all.

The second prothallial cell becomes flattened because of pressure due to the growth of the antheridium initial, and its nucleus becomes plano-convex or even meniscus-shaped, with its plane or concave face turned toward the first prothallial cell (*figs. 18, 20*). The nucleus of the antheridial cell, still at the center of the microspore, enlarges very much (*fig. 18*) and divides (*fig. 19*), giving rise to the generative

cell and the tube nucleus (*fig. 20*). The tube nucleus, although lying in close proximity to the wall of the microspore, does not become flattened as do the prothallial cells. In all preparations examined there seems to be no break in the cytoplasm surrounding the tube nucleus and the second prothallial cell (*figs. 20–22*), nor is a wall laid down between the tube nucleus and the primary spermatogenous cell. The primary spermatogenous cell—or generative cell—lies in a mass of cytoplasm differentiated from the surrounding cytoplasm by a slightly denser zone (*figs. 20, 21*). This condition of affairs is doubtless comparable to that of the generative cell of angiosperms, where there is a well-defined *Hautschicht*, on the outside of which food material is conspicuous. The primary spermatogenous cell dividing (*fig. 21*) gives rise to the stalk cell and the body cell, both of which lie within the cytoplasmic ring previously mentioned as surrounding the primary spermatogenous cell. The male gametophyte at this time (April 1, 1903) contains five nuclei: two prothallial cells, tube nucleus, stalk cell, and body cell. The microspore will be shed ten to fifteen days later.

The time periods in the development of the strobilus and male gametophyte are as follows: The strobilus appeared the previous season; on December 20, 1902, the group of cells which gives rise to the staminate flower is apparent, but the "perianth" is not yet visible; January 27, 1903, the "perianth" is well along and the primordia of the sporangia are clearly apparent; February 10, the primary wall cells are dividing; by February 15 many sporangia have mother-cells in the resting condition; one month later (March 15) the reduction division takes place; by April 1 the spores are mature, and about April 15 the pollen is shed. These records are for one season only, and the periods may be expected to vary somewhat in other seasons, the variations being of course dependent on various external factors.

It appears that Jaccard never saw the prothallial cells, for he says that at maturity the pollen grain contains three nuclei: "a large central nucleus representing the antheridial cell of Belajeff and of Strasburger; and two vegetative polar nuclei, of which one is the tube nucleus (*noyau du tube pollinique, Pollenschlauchkern*), and the other homologous with the *Stielzelle* of the German authors." So, putting this into present terminology, what he saw at the shedding of the pollen

were tube nucleus, stalk cell, and body cell. It is hardly to be expected that two prothallial cells will be present in one species and wholly absent in another.

The number of prothallial cells varies in gymnosperms. Those in which two have been reported are *Ginkgo*, Strasburger ('92); *Larix europaea*, Strasburger ('84); *Picea vulgaris*, Belajeff ('93); *Pinus Laricio*, Coulter and Chamberlain ('01); *Podocarpus coriacea*, Coker ('02); *Ceratozamia longifolia*, Juranyi ('82), sometimes two, but more often one. Those in which one has been reported are *Ceratozamia*, Juranyi ('82); *Zamia*, Webber ('97); *Cycas*, Ikeno ('98); *Ephedra campylopoda*, Strasburger ('72). No prothallial cells have been observed in *Biota*, *Cupressus*, and *Juniperus*, Strasburger ('92); *Taxus baccata*, *Juniperus*, Belajeff ('93); *Thuja occidentalis*, Land ('02); *Taxodium distichum*, Coker ('03); *Cupressus* (4 spp.), *Taxus baccata* and 4 vars., *Juniperus* (2 spp.), *Chamaecyparis* (5 spp.), *Callitris*, *Cryptomeria japonica*, and *Thuja orientalis*, Coker ('04); *Ephedra helvetica*, Jaccard ('94).

It is quite probable that in many gymnosperms two prothallial cells will be found eventually, and probably one at least will be found in some of those forms where up to the present none have been demonstrated.

THE OVULATE STROBILUS.

The ovulate strobilus was first collected in December. It differs in external appearance from the staminate strobilus in that it is longer and more slender.

The ovulate flower is not differentiated as early as is the staminate. On March 1, 1903, traces of the outer and inner integuments could be seen; a few days later the integuments presented the appearance shown in *fig. 23*. Much has been said concerning these integuments or perianths, as they are variously called. Transverse sections at different levels (*figs. 24, 25*) show that the outer integument results from a fusion of four leaves, and the inner integument from a fusion of two leaves. The outer integument becomes several cells thick, and in later stages quite hard. The inner integument is never more than two cells thick. A short time before the pollen is shed, the inner integument rapidly elongates and thrusts itself out through the apex of the strobilus. The exposed end is wide open, and is also

slit a short distance down one side (*fig. 44*). The pollen enters the open end of the integument, and drops down to the bottom of the pollen chamber (*fig. 44*), where it lies in contact with the archegonial end of the female gametophyte. So far as known, there is no other gymnosperm in which the pollen grain is placed so near the archegonia.

The archesporium could not be traced definitely back to a single hypodermal cell, but there are indications that such may be its origin. The earliest stage in which a suggestion of differentiation was observed is shown in *fig. 26*, March 8, 1903. The lower larger cell in this figure is beyond doubt the megaspore mother-cell. The large cell above will divide again and again, and thus place the megaspore mother-cell deeply within the nucellus. In *fig. 27* the divisions of a similar cell are clearly apparent, and the conspicuous megaspore mother-cell is shown. In general not more than one megaspore mother-cell is organized, but instances were noted in which two and very rarely three mother-cells were present. Sometimes, but not always, each of these cells produce megaspores. In general one mother-cell soon gains an advantage over the others and causes their rapid disintegration.

The mother-cell grows rapidly, meanwhile encroaching on the surrounding nucellar tissue. The reduction division occurred about March 8, 1903 (*fig. 28*). The second division quickly follows the first, and the more deeply placed megaspore alone functions. According to both Strasburger and Jaccard, three megaspores only are produced in the forms studied by them. In *E. trifurca* either three or four may occur (*figs. 29, 30*). In many instances the upper cell does not divide; again, the division may be incomplete, or it may be completed entirely. In no observed instance does the division of the upper cell take place until two lower megaspores are entirely separated; *fig. 29* shows such a late division of the upper cell. It seems that the more deeply placed cell because of its relation to the food supply is enabled to divide first. From this it follows that the most favorably placed megaspore—the lower one—is enabled to grow so rapidly as to preclude much further development on the part of the others. The megaspore remains a very short time in the resting condition. The number of chromosomes at the reduction division is

twelve, thus confirming the observations made on the microspore series.

Lang ('97) has shown that in Stangeria the mother-cell forms a row of three megaspores; Treub ('81) reports the same for Ceratozamia; and three are reported for Ginkgo. Among the Coniferales four are frequent. In *Pinus Laricio* I have observed that either three or four are formed indifferently. Strasburger ('79) gives three as the usual number in Taxus, although four frequently occur; in his later work on *Taxus baccata* (04') he says that four cells are formed from the megaspore mother-cell. Juel ('00) finds four in *Abies sibirica* and *Larix sibirica;* Shaw ('96) reports four in Sequoia; Lawson ('04) studying the same species of Sequoia finds three; Coker ('03) finds three in *Taxodium distichum* and ('04) four in *Thuja orientalis*, where they are not arranged in a row, but in nearly regular tetrad form. Strasburger finds three in *Ephedra campylopoda*, and Jaccard three in *E. helvetica;* in *E. trifurca* three or four are formed indifferently, dependent on the rapidity with which the functioning megaspore encroaches. This encroachment is probably the reason for the differences reported in the forms mentioned above.

THE FEMALE GAMETOPHYTE.

The two nuclei resulting from the division of the megaspore seem invariably to take the position with reference to the major axis of the ovule shown in *fig. 31*, for in no observed instance did the nuclear plate vary from this position. Before the spindle fibers and cell-plate have disappeared, a ring-like vacuole appears, entirely surrounding the cell-plate. The rapid increase in size of this vacuole is one of the chief factors concerned in the parietal placing of the free nuclei. These two nuclei divide simultaneously, and the resulting four take equidistant positions at the periphery of the embryo sac (*fig. 32*). Successive simultaneous divisions (*figs. 33, 35*) rapidly follow each other until the maximum number of nuclei is reached, which in the present instance apparently does not exceed 256. It may be of interest to note that at only one time—immediately after the division of the megaspore—is the vacuole free from cytoplasm. Careful staining shows that at all later stages (*figs. 32–35*) it is filled with a delicate cytoplasmic structure, which gradually increases in density until free nuclear division ceases, which was about April 1,

1903. Free nuclear division, therefore, extends through a period of approximately twenty days.

Simultaneously with the appearance of walls, the gametophyte is differentiated into two distinct regions: a micropylar or sex-organ producing region, and an antipodal or nutritive region. The behavior of the lower part of the gametophyte is strongly suggestive of the same region in *Gnetum Gnemon*, as described by Lotsy ('99). The cells of the antipodal region are only slightly elongated and are fairly regular in outline. As growth proceeds—and it is very rapid—this lower part is again separated into two physiologically distinct regions: storage and haustorial. The storage region comprises the greater part of the gametophyte, and is highly charged with starch and other foods. In the center are a few rows of thin-walled cells containing much more food than the surrounding cells, and extending up to the base of the archegonia. It is down through this thin-walled region that the embryo is thrust by the elongation of the suspensor. The haustorial part of the gametophyte (*fig. 44*) is composed of one or two layers of the outermost cells, which are clearly haustorial in function. Those at the tip of the gametophyte are elongated to a point ending in a single cell. The haustorial cells do not have the great elongation shown by the cells in the same region of Zamia. The storage and haustorial region increases in size as long as the embryo continues to grow.

The region in the immediate vicinity of the archegonia and for some distance below is very loosely organized, and the cell-walls are extremely delicate. In the central region immediately beneath the archegonia instances were noted in which the walls were late in appearing. The cells of this region are very vacuolate, and in consequence have little contents.

This feebly organized region is significant from a phylogenetic standpoint. In Tumboa the upper part of the gametophyte is loosely organized, and the numerous cells which function as eggs never get beyond the archegonium initial stage. In *Gnetum Gnemon* the same region never gets beyond the free nuclear stage, and these free nuclei function directly as eggs. It is possible that Ephedra, Tumboa, and Gnetum show stages through which the ancestral forms of angiosperms in all probability passed.

THE ARCHEGONIUM.

About April 1, 1903, the archegonium initials were first observed. They are the pyramidal form common to most gymnosperms. Two is the usual number, one is occasional, and three are rare. The primary neck-cell is quickly cut off after the initial becomes apparent (*fig. 36*), and almost immediately divides periclinally (*fig. 37*). Other periclinal walls follow (*fig. 40*), sometimes as many as four or five tiers being cut off before anticlinal walls appear; and as many as eight tiers of neck cells have been observed. Each tier divides anticlinally into four cells; later there may be six or eight in a tier, also the walls which come in later are no longer truly periclinal, thus giving the neck a somewhat irregular appearance (*fig. 41*). Thirty-two is probably the minimum number of cells, but it may go much higher. *Fig. 39* shows a cross section of the neck 40 μ above the top of the central cell. Of all gymnosperms Ephedra has the longest-necked archegonium. This may be due to the fact that the archegonial end of the gametophyte is freely exposed to the air.

Simultaneously with the appearance of the archegonium initials, a change is observable in the nucellus. Traces of disorganization become visible at the tip of the nucellus and gradually proceed downward, so that by the time the ventral nucleus is cut off, the cells at the apex of the nucellus have completely disappeared, leaving a pollen-chamber shaped like the frustrum of an inverted cone. The pollen-grains are thus enabled to come in direct contact with the gametophyte and the necks of the archegonia. So far as has been reported, Ephedra is the only gymnosperm having any part of the gametophyte exposed freely to the air, except in the case of *Cycas circinalis*, where, according to Warming ('77), if fertilization does not occur, the gametophyte continues to grow, ultimately bursting out through the micropyle and developing chlorophyll on exposure to light. Strasburger's figures show a pollen-chamber in *E. altissima*, but not in *E. campylopoda;* and Jaccard finds one present in *E. helvetica*. In Cycadales and Ginkgoales the pollen-chamber, formed by disintegration of the cells of the nucellar beak, is a conspicuous feature. In *Pinus Laricio* it is so small as to escape notice in most instances, while in *Thuja occidentalis* the tip of the nucellus at the time of pollination is an expanded stigma-like surface.

The nucleus of the central cell lies in close proximity to the neck of the archegonium. As the central cell enlarges, it does not have a conspicuous vacuole in the center, like Pinus and the Cupressineae, but is almost completely filled with cytoplasm except in the immediate vicinity of the nucleus, where there are a few small vacuoles. Later the cytoplasm in the lower part of the archegonium becomes almost homogeneous. A conspicuous kinoplasmic mass lies at a little distance below the nucleus (*fig. 41*). In the earliest stages it is coarsely granular, and later becomes dense, and is larger and sharper in outline than the similar body which is so conspicuous in some of the pines and in *Thuja occidentalis*.

When two or three archegonia are present, one is usually smaller than the others, as is shown in *fig. 38* (a cross-section through the middle region of the archegonia). When one archegonium is present it is very large as compared with the larger of two in a gametophyte. It is questionable if the eggs in the smaller archegonia regularly function.

The jacket-cells are at first rectangular, with the longer axis at right angles to the long axis of the central cell (*fig. 40*). Since periclinal division does not keep pace with the elongation of the central cell, the jacket-cells become much elongated (*fig. 41*). Their walls, never at any time thick, become so tenuous that they can scarcely be seen, and evidently offer little resistance to the passage of food into the central cell. There is evidence that at the time of fertilization the walls separating them from the egg break down altogether. *Fig. 41* shows two archegonia at the time of pollination (April 15, 1903).

The ventral nucleus was cut off about April 15 in the season of 1903. In material collected during the season of 1904 from the same plants, the ventral nucleus was cut off about April 1. This difference is probably due to the fact that the season of 1904 was unprecedentedly hot and dry.

No trace of a wall can be seen between the ventral nucleus and the egg, although in some instances there is a suggestion of cytoplasmic thickening between the two nuclei. The cytoplasm at the upper end of the central cell is still quite vacuolate; in the lower part it has now become very dense, in fact almost homogeneous.

The ventral nucleus remains in the upper part of the archegonium, and enlarging (*fig. 42*) becomes very conspicuous. The egg nucleus passes to the center of the archegonium, enlarges, and surrounds itself with a mass of cytoplasm, slightly different from that farther away from the nucleus in that it at first consists of radial strands proceeding out from the nucleus in all directions. A thickening of the cytoplasm next appears all around the nucleus at the place where the radiations meet the general cytoplasm of the archegonium. This thickening is very pronounced in most instances, again it can be seen with difficulty; it very much resembles the first appearance of the membrane around the egg and synergids of angiosperms.

SUMMARY.

Ephedra trifurca is monosporangiate, but bisporangiate strobili occasionally occur.

The beginnings of the staminate flower were clearly apparent in December, and the pollen was shed about the middle of April, the interval being thus a little over four months.

The anthers develop in acropetal succession on a strobilus, and are surrounded by a perianth.

Microspore mother-cells were observed about the middle of February, and the reduction division occurs about one month later.

The gametophyte number of chromosomes is twelve.

There are two persistent prothallial cells; the first is cut off by a wall; the second is not cut off by a wall.

The primary spermatogenous cell surrounds itself by a membrane (*Hautschicht?*), and on the division of the primary spermatogenous cell, the stalk cell and body cell continue to be surrounded by this membrane, and are not separated from each other by a wall.

The only wall formed in the pollen grain is the one which cuts off the first prothallial cell.

The male gametophyte at the time of shedding consists of two prothallial cells, stalk cell, body cell, and tube nucleus.

The megasporangium is surrounded by two integuments, the outer of which consists of four fused leaves; the inner of two fused leaves.

The megaspore mother-cell is deeply placed within the nucellus

and gives rise to either three or four megaspores arranged in a row, the most deeply placed megaspore being functional.

The nuclei resulting from the division of the megaspore show polarity in that they are definitely oriented with respect to the axis of the megasporangium.

A vacuole appears between the nuclei resulting from the division of the megaspore before the spindle has disappeared, and soon becomes filled with delicate cytoplasmic structures which increase in density until walls appear.

The free nuclei are parietally placed from the beginning, divide simultaneously, and are presumably 256 in number before walls appear.

The female gametophyte is separated into two regions: a loosely formed archegonial region, and a more compact antipodal region, the latter being composed of a haustorial and a storage region.

The archegonia vary from one to three, two being the usual number; the neck is composed usually of eight tiers of cells; and there are no archegonial chambers.

The apex of the nucellus breaks down, and a conspicuous pollen-chamber is formed. The necks of the archegonia are thus exposed to the air, and the microspores are brought directly into contact with the female gametophyte.

No wall is formed between the ventral nucleus and the egg; the former becomes quite large and takes a position a short distance below the neck of the archegonium.

The egg takes a position midway in the cytoplasm of the archegonium, surrounds itself with a membrane comparable to the one which invests the eggs of angiosperms, and in this position awaits fertilization.

At the time of fertilization the cytoplasm in the archegonium has become almost homogeneous and very dense, except in the region immediately below the neck of the archegonium, where it is loosely vacuolate.

Thanks are due Professor John M. Coulter and Dr. Charles J. Chamberlain for criticism and advice; also Mr. O. B. Metcalfe, Mesilla Park, N. M., for efficient collecting of material.

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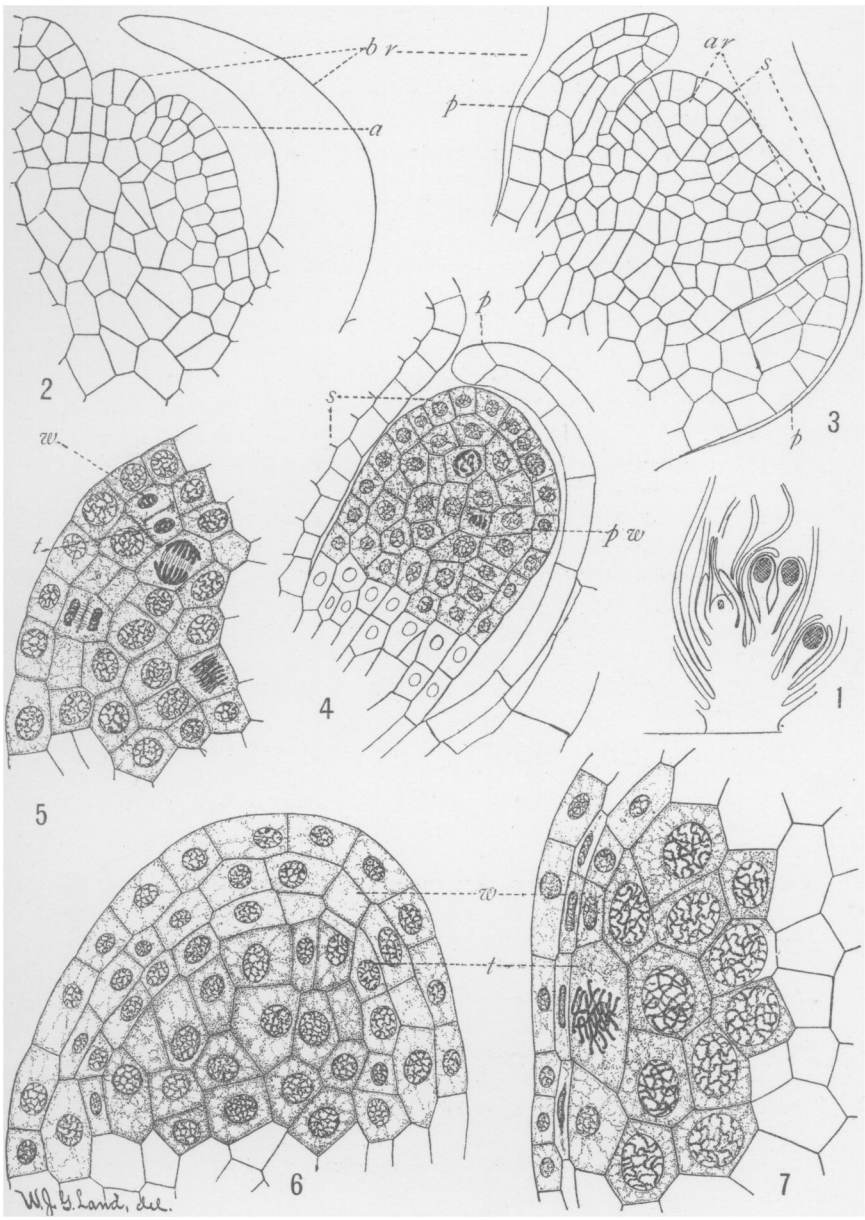

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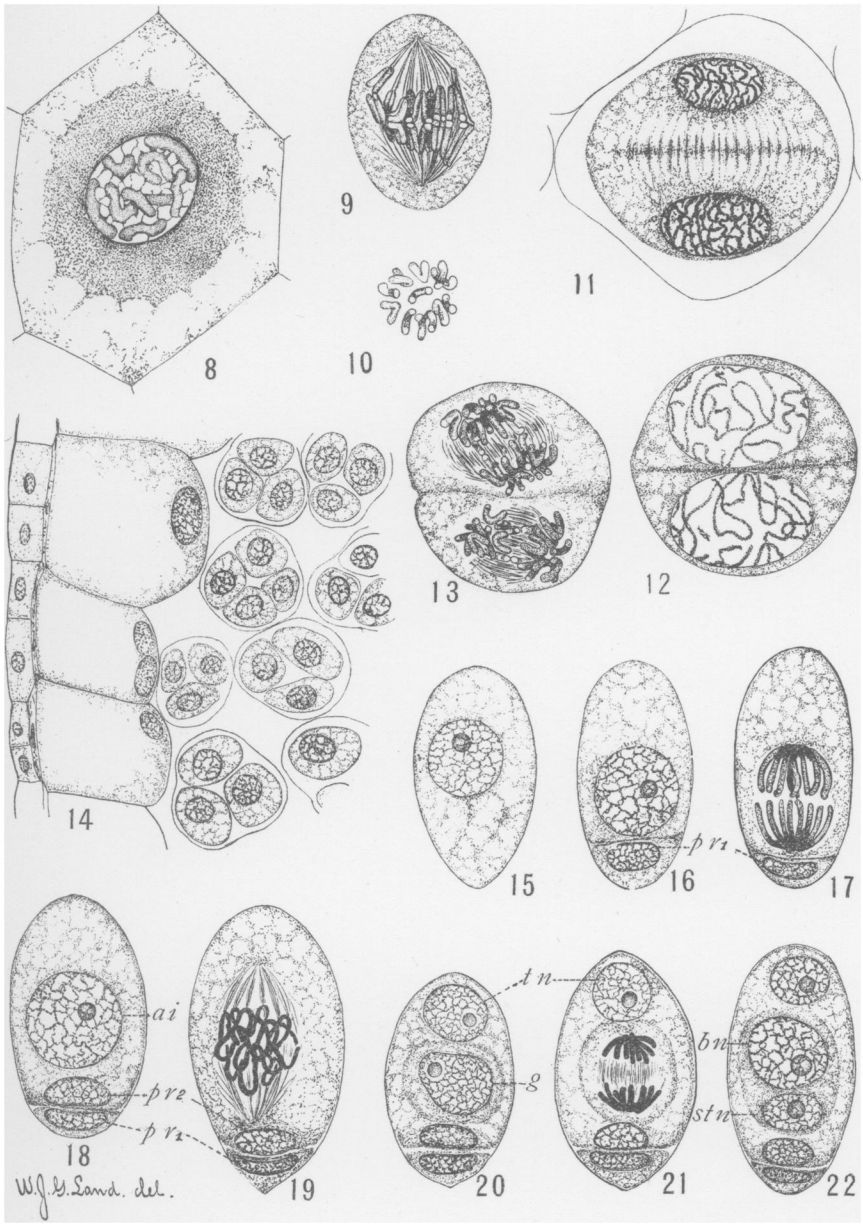

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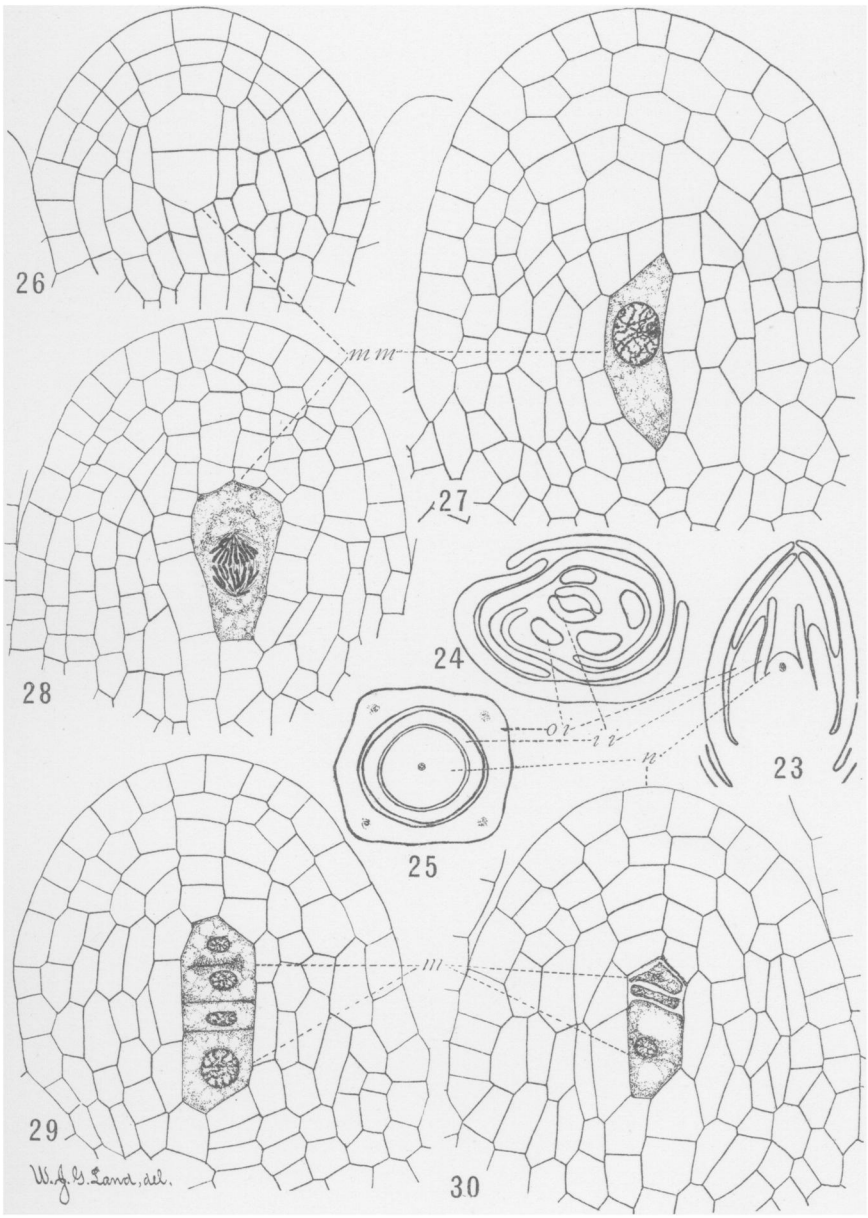

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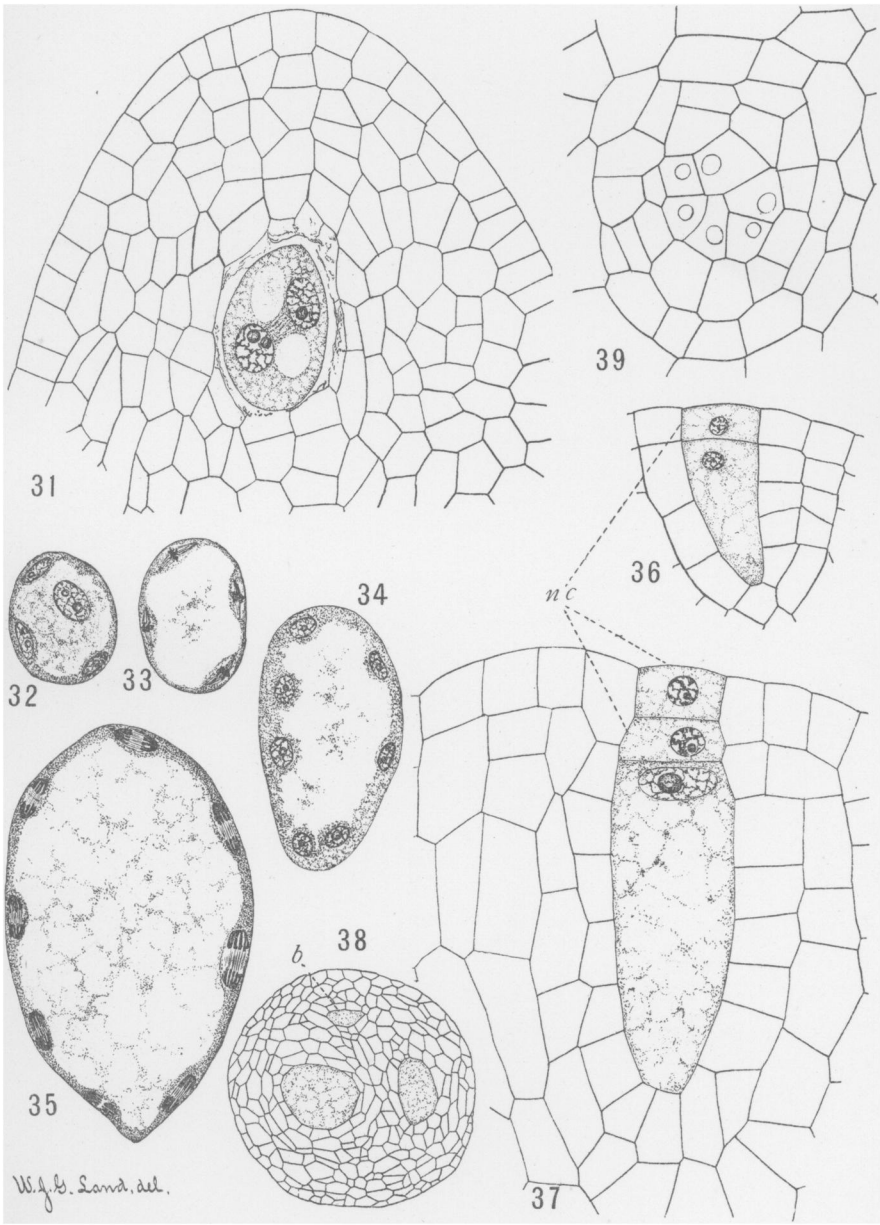

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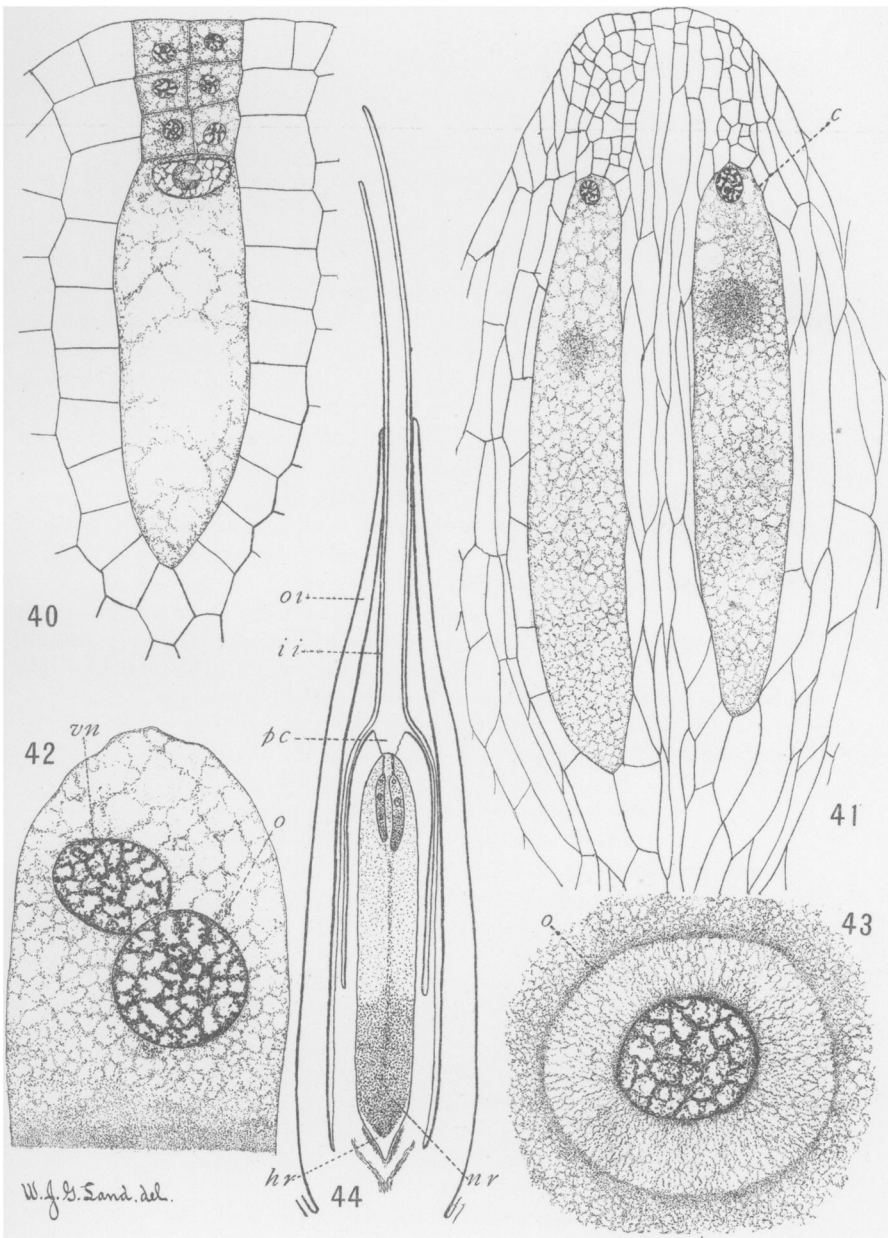

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EXPLANATION OF PLATES I–V.

All figures were drawn with an Abbé camera lucida, and reduced one-half in reproduction. Abbreviations are: *br*, bract; *a*, primordium of stamen; *p*, perianth; *oi*, outer integument; *ii*, inner integument; *n*, nucellus; *pc*, pollen-chamber; *s*, microsporangium; *pw*, primary wall-cells; *w*, wall-cells; *t*, tapetum; *ar*, archesporial cells; *pr*^{1}, first prothallial cell; *pr*^{2}, second prothallial cell; *ai*, antheridium initial; *tn*, tube nucleus; *g*, primary spermatogenous cell; *stn*, nucleus of stalk cell; *bn*, nucleus of body cell; *mm*, megaspore mother-cell; *m*, megaspores; *nr*, nutritive region of female gametophyte; *hr*, haustorial region of gametophyte; *b*, abortive archegonium; *nc*, neck cells; *c*, central cell; *vn*, ventral nucleus; *o*, egg.

FIG. 1. A bisporangiate strobilus. × 16.

FIG. 2. Portion of a longitudinal section through a staminate strobilus showing bracts and primordia of two anthers. × 225.

FIG. 3. Longitudinal section through an anther showing beginning of perianth and primordia of two sporangia. × 225.

FIG. 4. Longitudinal section through a sporangium showing sporogenous cells, part of an adjoining sporangium, and perianth. × 225.

FIG. 5. Division of primary wall cells; the sporogenous cells are also actively dividing. × 500.

FIG. 6. Early stage of the microspore mother-cells. × 500.

FIG. 7. Microspore mother-cells in resting stage. × 500.

FIG. 8. Microspore mother-cell after segmentation of the spirem. × 1500.

FIG. 9. Microspore mother-cell, heterotypic division. × 1500.

FIG. 10. Polar view of chromosomes in anaphase of second mitosis in microspore mother-cell. × 1500.

FIG. 11. Later stage in division of microspore mother-cell. × 1500.

FIG. 12. Prophase of homotypic division. × 1500.

FIG. 13. Homotypic division. × 1500.

FIG. 14. Tetrads and enlarged tapetal cells. × 500.

FIG. 15. A microspore shortly before the first division. × 1500.

FIG. 16. Microspore after formation of first prothallial cell. × 1500.

FIG. 17. Division to form second prothallial cell and antheridium initial. × 1500.

FIG. 18. First and second prothallial cells and antheridium initial. × 1500.

FIG. 19. Division of antheridium initial. × 1500.

FIG. 20. Two prothallial cells, primary spermatogenous cell, and tube nucleus. × 1500.

FIG. 21. Division of primary spermatogenous cell. × 1500.

FIG. 22. The two prothallial cells, stalk cell, body cell, and tube nucleus. × 1500.

FIG. 23. Median longitudinal section through a megasporangiate strobilus showing the two integuments and nucellus with gametophyte. × 46.

FIG. 24. Section through the strobilus above the nucellus showing the four parts of the outer integument and the two parts of the inner integument which later become tubes. × 46.

FIG. 25. Section through the strobilus at level of gametophyte, showing the fused parts. × 46.

FIG. 26. Megaspore mother-cell becoming differentiated. × 500.

FIG. 27. Megaspore mother-cell, resting stage. × 500.

FIG. 28. Megaspore mother-cell dividing. × 500.

FIG. 29. A row of four megaspores, showing late division of upper daughter nucleus. × 500.

FIG. 30. A row of three megaspores; the upper daughter-nucleus has failed to divide. × 500.

FIG. 31. First division of the megaspore, showing formation of the central vacuole. × 500.

FIG. 32. Four-celled stage of female gametophyte. × 500.

FIG. 33. Simultaneous division of the nuclei; eight-celled stage. × 500.

FIG. 34. Female gametophyte; sixteen cells. × 500.

FIG. 35. Female gametophyte; simultaneous division to form sixty-four free nuclei. × 500.

FIG. 36. Archegonium with primary neck cell and central cell. × 500.

FIG. 37. Archegonium showing the enlarged central cell and two neck cells. × 500.

FIG. 38. Transverse section of gametophyte at level of central cell, showing a large, a small, and an abortive archegonium. × 75.

FIG. 39. Transverse section through neck of an archegonium at a distance of 40 μ above the central cell. × 500.

FIG. 40. An archegonium slightly older than *fig. 37*. × 500.

FIG. 41. Two archegonia just before the division of the nucleus of the central cell. × 112.

FIG. 42. Egg nucleus and ventral nucleus lying in the upper part of the archegonium. × 500.

FIG. 43. Egg lying near the center of archegonium and surrounded by a membrane of thickened cytoplasm, and ready for fertilization. × 500.

FIG. 44. Longitudinal median section through the ovule and integuments, showing reproductive, storage, and haustorial regions of the gametophyte × 48.